

**PATENT COOPERATION TREATY**

73776-139 DES:jbm:pdz

NATIONAL RESEARCH COUNCIL OF CANADA  
(W. A. Keller, et al)

Serial No. PCT/CA99/01223  
Filed: December 22, 1999

**TRANSGENIC PLANTS COMPRISING A CONDITIONALLY LETHAL GENE**

To: International Preliminary Examining Authority  
European Patent Office  
D-80298 Munich  
Germany

Dear Sir:

**REPLY TO WRITTEN OPINION AND AMENDMENT  
PURSUANT TO ARTICLE 34 PCT**

This is responsive to the Written Opinion mailed on November 9, 2000 in the subject case. Please amend the application as follows, without prejudice or disclaimer:

**IN THE CLAIMS**

Please replace pages 47-53 of record, containing claims 1-59, with new pages 47-53 containing claims 1-58, enclosed herewith.

**REMARKS**

There are now 58 claims standing. Claim 1 has been cancelled, and claims 2-16 of record have been renumbered as claims 1-15, accordingly. New claim 1 specifies that the second gene, when expressed in a plant, confers a novel trait on the plant. The last clause in claim 1 has been deleted, as the presence of a promoter is inherent in the phrase "adapted for expression in a plant cell."

Claim 2 now specifies that the second gene is heterologous to the plant. A typographic error has been corrected in claim 14 as presently amended (claim 15 of record). The claim now specifies "an oncogene under the control of a promoter of a low temperature inducible gene from Arabidopsis." Support for this amendment is found in the specification at, for instance, page 26, lines 13-15. New claim 16 specifies that the conditionally lethal gene is the gene coding for rhizobitoxine synthase or the gene coding for methoxinined dehydrogenase.

Claims 17-55, as presently amended, correspond to claim 17-55 of record. Claims 17-19 are amended to clarify that the promoter specified is that of the conditionally lethal first gene. Claim 24 is amended to specify preceding claims in the alternative only. Claim 49 is amended to delete the words "during transformation" from the preamble.

Claim 56 is amended to incorporate the substance of claim 57 of record. Claim 57 of record is cancelled, and claims 58 and 59 of record are renumbered as claims 57 and 58 accordingly.

Applicant respectfully submits that the amendments are fully supported by the specification and claims as originally filed, and therefore do not constitute new matter.

The IPEA contends that each of documents D1 to D4 discloses a genetic construct comprising a conditionally lethal gene operably linked to a promoter, and that the gene is either oncogene 2 (iamH) or phosphonate monoester hydrolase. The IPEA further contends that at least the constructs disclosed in D1 appear to comprise the iamH gene and a second gene (ampicillin resistance). The IPEA concludes that the subject matter of claims 1-3, 10, 11, 13, 15, 16, 18 and 20-24 therefore lacks novelty pursuant to Article 33(2) PCT.

Applicant respectfully traverses these rejections. The claims as presently amended patentably distinguish from D1 to D4. Claim 1 as presently amended is directed to a genetic construct comprising: a conditionally lethal first gene adapted for expression in a plant cell; and, a second gene adapted for expression in a plant cell; said second

gene, when expressed in a plant, conferring a novel trait on the plant. None of D1 to D4, or any combination of them, teaches or suggests a genetic construct comprising a conditionally lethal first gene adapted for expression in a plant cell and a second gene which confers a novel trait on a plant, adapted for expression in a plant cell.

The IPEA contends that the constructs disclosed in D1 appear to comprise the iamH gene and a second gene, being an ampicillin resistance gene. Applicant respectfully submits that the construct described in D1 comprising the iamH gene and an ampicillin resistance gene was prepared solely for purposes of in vitro manipulation of DNA, and not for expressing the ampicillin gene in the plant. Indeed, it appears from columns 31 through 33 of D1 that the ampicillin gene was present only for the purpose of selecting transformed bacteria. There is no teaching or suggestion that the ampicillin gene was adapted for expression in a plant as claimed in presently amended claim 1. Further, one of skill in the art would not consider an ampicillin gene, present only to aid in DNA manipulation steps, to be a gene which confers a "novel trait" on a plant, in the context of the invention. Novel traits are discussed in the specification at pages 1-4. It is respectfully submitted that D1 does not teach or suggest a second gene which encodes a "novel trait" with in the meaning of that term in the instant specification and claims.

Applicant therefore respectfully submits that the subject matter of claim 1 is novel and inventive over D1 to D4 and therefore complies with Articles 33(2) and 33(3) PCT. Claims 3, 10, 11, 13, 15, 16, 18 and 20-24 of record (corresponding to claims 2, 9, 10, 12, 14, 15, 18 and 20-24 as presently amended) are all ultimately dependent on claim 1 as presently amended, and the subject matter of these claims is therefore also necessarily novel and inventive over the teachings of D1 to D4. Reconsideration and withdrawal of these rejections are therefore respectfully requested.

The IPEA further contends that D1 anticipates the methods of claims 28, 29, 31, 32 and 36 of record, and that D3 anticipates the methods of claims 30 and 33-35 of record. These claims, which maintain the same claim numbering in the presently amended claims, are all ultimately dependent on presently amended claim 1 which specifies a second gene adapted for expression in a plant cell, and which, when expressed in a plant,

confers a novel trait on the plant. Applicant respectfully submits that this feature is not disclosed or suggested by either D1 or D3. Reconsideration and withdrawal of the rejections of these claims are therefore respectfully requested.

Claims 12, 14, 17, 19, 25-27 and 37-39 stand rejected as not being based on an inventive activity, and therefore lacking compliance with Article 33(3) PCT. Applicant respectfully submits that all of these claims are ultimately dependent from claim 1 as presently amended. As claim 1, as presently amended, is both novel and inventive over the cited prior art, so must be claims 12, 14, 17, 19, 25-27 and 37-39. Reconsideration and withdrawal of the rejection of these claims are therefore respectfully requested.

The IPEA contends that the callusing medium disclosed in D5 contains NAA and that D5 thus anticipates the subject matter of claim 56. The IPEA also contends that the use of callusing medium comprising NAA disclosed in D5 is not linked to any particular Brassica variety or phenotype. The IPEA contends that the subject matter of claims 57 and 58 of record thus is not considered to be based on an inventive activity in the sense of Article 33(3) PCT. Applicant respectfully submits that claims 56 and 57 as presently amended patentably distinguish from D5. Claim 56 now specifies that the Brassica napus has altered oil profile. Claim 57 specifies that the Brassica napus is variety AG-019. D5 discloses only the use of NAA in a callusing media for the cultivar "Profit" and makes no mention of the use of NAA in callusing media for Brassica transformation generally. Brassica cultivars respond differently to culture conditions, and those of ordinary skill in the art would not conclude from D5 that the inclusion of NAA in the media at the callusing and recovery step would have utility in Brassica cultivars other than "Profit", the one specific cultivar disclosed by D5. D5 does not teach or suggest that NAA would be useful in Brassica napus cultivars having altered oil profile or for the variety AG-019, as claimed in presently amended claims 56 and 57. Reconsideration and withdrawal of the rejections of presently amended claims 56 and 57 are therefore respectfully requested.

The IPEA contends that claim 3 (claim 2 as presently amended) is not clear with respect to what said gene should be heterologous to. Claim 2 is amended to clarify that the second gene is heterologous to said plant cell.

The IPEA contends that claim 49 is unclear for inclusion of the expression "during transformation." Claim 49 is amended to delete this expression.

The IPEA contends that claim 59 of record (claim 58 as presently amended) identifies plasmids by trivial names which are meaningless for the skilled person and thus not suitable to define the claimed subject matter. Applicant respectfully submits that the identity of the claimed plasmids is clear from the specification as a whole, which describes in detail the construction of each of the claimed plasmids. In particular, the construction of the claimed plasmids is illustrated in Figures 1-6, and in the Examples contained in the specification. Applicant therefore respectfully submits that the identity of each of these plasmids is clear to the skilled person.

As noted by the IPEA, several claims refer to genes "adapted for expression in a plant cell". The IPEA contends that the application does not appear to provide guidance on how adapted genes could be obtained. Applicant respectfully notes that suitable genes adapted for expression in a plant cell are described in the application at pages 23 and 24.

In view of the foregoing, Applicant respectfully requests entry of the amendment and reconsideration and withdrawal of the rejections contained in the Written Opinion. Issuance of an International Preliminary Examination Report containing a favourable finding as to the novelty, inventive step, and industrial applicability of all of claims 1-58 as presently amended are respectfully requested.

Yours very truly,

SMART & BIGGAR

Ottawa, Canada

Encl.

(Mrs.) Joy D. Morrow

February 1, 2001

CLAIMS:

1. A genetic construct comprising:

a conditionally lethal first gene adapted for expression in a plant cell; and

5 a second gene adapted for expression in a plant cell, said second gene, when expressed in a plant, conferring a novel trait on said plant.

2. The construct of claim 1, wherein the second gene is heterologous to said plant cell.

10 3. The construct of claim 2, wherein the heterologous gene codes for a pharmaceutical product.

4. The construct of claim 2, wherein the heterologous gene codes for an industrially useful enzyme.

15 5. The construct of claim 2, wherein the heterologous gene codes for rennin and/or hirudin.

6. The construct of claim 1, wherein the second gene, when expressed, changes the phenotype of the plant.

7. The construct of claim 1, wherein the second gene, codes for a protein, peptide or anti-sense RNA.

20 8. The construct of claim 1, wherein the second gene codes for an input or output trait.

9. The construct of claim 1, wherein the conditionally lethal gene is an oncogene.

25 10. The construct of claim 1, wherein the conditionally lethal gene is oncogene 2 from *Agrobacterium tumefaciens*.

11. The construct of claim 1, wherein the conditionally lethal gene is expressed in response to chemical or stress.

12. The construct of claim 1, wherein the conditionally lethal gene is lethal only when an exogenous substance is applied.

13. The construct of claim 1, wherein the conditionally lethal gene is lethal when it is expressed and no exogenous substance need be applied.

14. The construct of claim 1, wherein the conditionally lethal gene is oncogene 4, an oncogene under the control of a promoter of a low temperature inducible gene from Arabidopsis, the gene coding for methoxinine dehydrogenase, the gene coding for rhizobitoxine synthase, or the gene coding for phosphonate monoester hydrolase.

15. The construct of claim 12, wherein the conditionally lethal gene is the gene coding for methoxinine dehydrogenase, the gene coding for rhizobitoxine synthase, or the gene coding for phosphonate monoester hydrolase.

16. The construct of claim 12, wherein the conditionally lethal gene is the gene coding for methoxinine dehydrogenase or the gene coding for rhizobitoxine synthase.

17. The construct of claim 1, wherein the promoter of said conditionally lethal first gene is inducible.

18. The construct of claim 1, wherein the promoter of said conditionally lethal first gene is tissue-specific.

19. The construct of claim 1, wherein the promoter of said conditionally lethal first gene is constitutive.

20. A plant transformation vector comprising the genetic construct of any one of claims 1 to 19.

21. A plant comprising the genetic construct of any one of claims 1 to 19.

22. A plant comprising the genetic construct of any one of claims 11, 12, 13 and 15.

5 23. A plant transformed with the vector of claim 20.

24. The plant of any one of claims 21 to 23 which is Brassica.

25. The Brassica plant of claim 24 which has altered oil composition.

10 26. The Brassica plant of claim 25 which has high oleic, low linoleic acid genotype.

27. The Brassica plant of claim 26 which is variety AG-019 or derivatives thereof.

15 28. A method for producing a transgenic plant which can be removed from a growing environment, comprising:

transforming a plant cell with the genetic construct or vector of any one of claims 1 to 20; and

regenerating the plant cell to a whole plant.

20 29. A method for removing the plant of claim 22 from a growing environment, comprising application of a chemical agent which is converted to a phytotoxic agent by a product of a conditionally lethal gene, wherein the agent is applied at a level which, upon conversion by the gene product, results in a sub-lethal level of converted substrate.

25 30. A method for visual identification of the plant of claim 22, comprising:



application of a chemical agent which is a substrate of the product of the conditionally lethal gene, wherein the agent is applied at a level which, upon conversion by the gene product, results in a sub-lethal level of converted substrate;

5 visually identifying the plants which manifest the sub-lethal phenotype.

31. The method of claim 29 or claim 30 wherein the genetic construct or vector comprises oncogene 2 as the conditionally lethal gene, and wherein the chemical agent is an indoleamide or a related derivative.

32. The method of claim 31 wherein the indoleamide is naphthalene acetamide.

33. A method for selecting a transgenic plant of claim 22, comprising:

15 application of a chemical agent which is a substrate for the product of the conditionally lethal gene, wherein the agent is applied at a level which, upon conversion by the gene product, results in a sub-lethal level of converted substrate;

20 visually identifying the plants which manifest the sub-lethal phenotype; and

allowing the identified plants to recover into normal plants in the absence of the chemical agent.

34. The method of claim 33 wherein the genetic construct or vector comprises oncogene 2 as the conditionally lethal gene, and wherein the chemical agent is an indoleamide or a related derivative.

35. The method of claim 34 wherein the indoleamide is naphthalene acetamide.

36. The method of any one of claims 29 to 35 wherein the plant is Brassica.

37. The method of claim 36 wherein the Brassica plant has altered oil composition.

5 38. The method of claim 37 wherein the Brassica plant has high oleic acid, low linoleic acid content.

39. The method of claim 38 wherein the Brassica plant is variety AG-019 or derivatives thereof.

40. A method for visual identification of a germinating seed or plant embryo comprising oncogene 2 as a transgene, comprising:

culturing the seed or embryo on a medium containing an indoleamide or a related derivative; and

visually identifying the germinated seed or embryo which manifests the phenotype.

41. A method for selecting a germinating seed or plant embryo comprising oncogene 2 as a transgene, comprising:

culturing the seed or embryo on a medium containing an indoleamide or a related derivative;

visually identifying the germinated seed or embryo which manifest the phenotype; and

transferring the identified seed or embryo to a medium without indoleamide;

thereby obtaining the germinating seed or plant embryo comprising oncogene 2 as a transgene.

42. The method of claim 40 or 41 wherein the medium of step (a) contains an auxin transport inhibitor and the medium of step (b) does not contain an auxin transport inhibitor.

43. The method of any one of claims 40 to 42, wherein the inhibitor is N-(1-naphthyl)phthalamic acid; 2,3,5-triiodobenzoic acid; 9-hydroxyfluorene-9-carboxylic acid; erythrosine; eosine; fluorescein; semicarbazone; or ethanphon.

44. The method of any one of claims 40 to 43, wherein the indoleamide is naphthalene acetamide and the inhibitor is naphthylphthalamic acid.

45. The method of any one of claims 40 to 44, wherein the seed or embryo is Brassica.

46. The method of claim 45, wherein the Brassica seed or embryo has altered oil composition.

47. The method of claim 46, wherein the Brassica seed or embryo has high oleic acid, low linoleic acid content.

48. The method of claim 47, wherein the Brassica seed or embryo is variety AG-019 or derivatives thereof.

49. A method for selecting a transgenic plant cell comprising:

transforming a plant cell with a genetic construct or vector comprising an oncogene adapted for expression in a plant cell;

exposing said plant cell to a formula comprising a benign auxin derivative of a plant hormone, which is converted into an active hormone by the product of the oncogene, and an auxin transport inhibitor;

culturing the cell to form a group of cells;

visually identifying the group of cells which manifests the phenotype associated with the active hormone; and,

allowing the identified group of cells to recover in the absence of the derivative.

50. The method of claim 49, wherein the oncogene is oncogene 2.

51. The method of claim 49, wherein the benign derivative is naphthalene acetamide and the inhibitor is naphthylphthalamide.

52. The method of any one of claims 49 to 51, wherein the plant cell is Brassica.

53. The method of claim 52 wherein the Brassica plant cell has altered oil composition.

54. The method of claim 53 wherein the Brassica plant cell has high oleic acid, low linoleic acid content.

55. The method of claim 54 wherein the Brassica plant cell is variety AG-019 or derivatives thereof.

56. A method for transforming Brassica napus, comprising inclusion of naphthalene acetic acid in the media at the callusing and recovery step, wherein the Brassica napus has altered oil profile.

57. The method of claim 56 wherein the Brassica napus is variety AG-019.

58. A plasmid selected from the group consisting of: pJH121, pJH122, pJH123, pJH125, pJH126, and pJH130.

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : W.A Keller, et al.  
Appln. No. : Continuation of PCT/CA99/01223  
Filed: : December 22, 1999  
Title : Transgenic Plants and Methods for Production Thereof  
Grp./A.U. :

Honorable Commissioner for Patents  
Washington, D.C. 20231

**AMENDMENT**

Sir:

By way of a Preliminary Amendment, please amend the above-identified application as follows:

**In the Specification:**

At page 1, after the title, please add the following new paragraph:

**--CROSS-REFERENCE TO RELATED APPLICATIONS**

This is a continuation of International Application No. PCT/CA99/01223, having an international filing date of December 22, 1999, and which claims priority from United States Provisional Patent Application Serial No. 60/113,546 filed on December 22, 1998, both of which are incorporated herein by reference.--

**In the Claims:**

Please replace claims 1-58 of record with new claims 59-103 attached hereto.

**REMARKS**

Claims 1-58 of record are cancelled. New claims 59-103 are added.

The claims of the international application contained multiple-dependent claims, which were dependent on other multiple-dependent claims. The new claims are amended to define the invention with greater clarity and to conform the claim dependencies with United States practice. Applicants respectfully submit that the new

claims are fully supported by the original specification and claims and that the amendments therefore do not constitute new matter.

Respectfully submitted,

LORUSSO & LOUD

BY \_\_\_\_\_

George A. Loud  
Reg. No. 25,814

June 22, 2001

LORUSSO & LOUD  
3137 Mount Vernon Avenue  
Alexandria, VA 22305

(703) 739-9393

CLAIMS:

59. A genetic construct, comprising:

a conditionally lethal first gene expressible in a plant cell of a plant; and

5 a second gene expressible in said plant cell, said second gene, when expressed in said plant cell, conferring a non-naturally occurring trait of interest on said plant cell.

60. The genetic construct of claim 59, wherein the second gene comprises nucleic acid which is heterologous to said plant cell.

61. The genetic construct of claim 60, wherein the second gene codes for a pharmaceutically active compound.

62. The genetic construct of claim 60, wherein the second gene codes for an industrially useful enzyme.

15 63. The genetic construct of claim 60, wherein the second gene codes for rennin and/or hirudin.

64. The genetic construct of claim 59, wherein the second gene, when expressed, produces a measurable change in a phenotype of said plant cell.

20 65. The genetic construct of claim 59, wherein the second gene codes for one of a protein, peptide or an anti-sense RNA.

66. The genetic construct of claim 59, wherein the second gene codes for an input or output trait conferrable on said plant cell.

25 67. The genetic construct of claim 59, wherein the conditionally lethal gene is an oncogene.

68. The genetic construct of claim 59, wherein the conditionally lethal gene is oncogene 2 from *Agrobacterium tumefaciens*.

69. The genetic construct of claim 59, wherein the  
5 conditionally lethal gene is adapted to be expressed in said particular plant in response to a chemical or physiological stress applied to said plant cell.

70. The genetic construct of claim 59, wherein the  
10 conditionally lethal gene is configured to express a gene product lethal to said particular plant upon application of a particular exogenous substance to said plant cell.

71. The genetic construct of claim 59, wherein the  
15 conditionally lethal gene is selected from the group consisting of oncogene 4 under the control of a promoter of a low temperature inducible gene from *Arabidopsis*, a gene coding for methoxinine dehydrogenase, a gene coding for rhizobitoxine synthase, and a gene coding for phosphonate monoester hydrolase.

72. The genetic construct of claim 59, further comprising  
20 an inducible promoter in operable association with said conditionally lethal first gene.

73. The genetic construct of claim 59, further comprising a tissue-specific promoter in operable association with said conditionally lethal first gene.

25 74. A plant transformation vector comprising the genetic construct of claim 59.

75 79. A plant transformation vector comprising the genetic construct of claim 59.

76 80. A transgenic plant, comprising:



a conditionally lethal first gene expressible in a plant cell of said transgenic plant; and

a second gene expressible in said plant cell of said transgenic plant, said second gene, when expressed in said plant cell, conferring a non-naturally occurring trait of interest on said plant cell.

81. The transgenic plant of claim 80, wherein said transgenic plant is a variety of Brassica.

82. The transgenic plant of claim 81, wherein said second gene confers upon said variety of Brassica a high oleic, low linoleic acid genotype.

83. The transgenic plant of claim 81, wherein said variety of Brassica is variety AG-019 or derivatives thereof.

84. A method for selectively removing at least one plant from a growing environment, comprising:

transforming at least one plant cell with a genetic construct including:

a conditionally lethal first gene expressible in said at least one plant cell; and

a second gene expressible in said at least one plant cell, said second gene, when expressed in said at least one plant cell, conferring a non-naturally occurring trait of interest on said at least one plant cell;

regenerating the at least one plant cell to at least one whole plant; and

applying a chemical agent to said at least one whole plant, said chemical agent being configured to be converted

into a phytotoxic agent of said at least one whole plant by one or more gene products of said conditionally lethal gene.

85. The method of claim 84, wherein said applying said chemical agent comprises applying said chemical agent in an amount selected to effect a sub-lethal level of said phytotoxic agent in said at least one whole plant upon said conversion by said one or more gene products of said conditionally lethal gene.

86. The method of claim 85, further comprising visually identifying a sub-lethal phenotype of said at least one whole plant.

87. The method of claim 84, wherein the genetic construct comprises oncogene 2 as the conditionally lethal gene, and wherein the chemical agent comprises an indoleamide or a related derivative.

88. The method of claim 87, wherein the indoleamide is naphthalene acetamide.

89. The method of claim 84, wherein the at least one whole plant is a variety of Brassica.

90. The method of claim 89, wherein said second gene confers upon said variety of Brassica a high oleic, low linoleic acid genotype.

91. The method of claim 90, wherein the variety of Brassica plant is variety AG-019 or derivatives thereof.

92. A method for selecting a germinating seed or plant embryo comprising oncogene 2 as a transgene, comprising:

providing at least one transgenic plant cell of a plant seed or plant embryo, said at least one transgenic plant cell including oncogene 2 as a transgene;

culturing the at least one transgenic plant cell on a medium comprising an indoleamide or a related derivative; and

visually identifying the at least one transgenic plant cell by its expression of an auxin-overproduction phenotype.

93. The method of claim 92, wherein said medium further comprises an auxin transport inhibitor.

94. The method of claim 93, wherein the auxin transport inhibitor is selected from the group consisting of N-(1-naphthyl)phthalamic acid, 2,3,5-triiodobenzoic acid, 9-hydroxyfluorene-9-carboxylic acid, erythrosine, eosine, fluorescein, semicarbazone, and ethanphon.

95. The method of claim 92, wherein the indoleamide is naphthalene acetamide and the auxin transport inhibitor is naphthylphthalamic acid.

96. The method of claim 92, wherein the at least one plant cell comprises a seed or a plant embryo.

97. The method of claim 92, wherein the at least one plant cell is derived from a variety of Brassica.

98. The method of claim 97, wherein the variety of Brassica is a variety having a high oleic acid, low linoleic acid profile.

99. The method of claim 97, wherein the variety of Brassica is variety AG-019 or derivatives thereof.

100. The method of claim 92, further comprising transferring the at least one transgenic plant cell to a second medium free from indoleamide and recovering the at least one transgenic plant cell.

97  
101. The method of claim 100, wherein the second medium comprises naphthalene acetic acid.

98  
102. The method of claim 92, further comprising transforming at least one plant cell with oncogene 2 to obtain  
5 said at least one transgenic plant cell.

99  
103. A method for producing a transgenic plant comprising oncogene 2 as a transgene, comprising:

providing at least one transgenic plant cell of a plant seed or plant embryo, said at least one transgenic plant  
10 cell including oncogene 2 as a transgene;

culturing the at least one transgenic plant cell on a medium comprising naphthalene acetamide and an auxin transport inhibitor;

visually identifying the at least one transgenic  
15 plant cell by its expression of an auxin-overproduction phenotype; and

transferring the at least one transgenic plant cell to a second medium comprising naphthalene acetic acid to recover the at least one transgenic plant cell.